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J Occup Environ Hyg. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

J Occup Environ Hyg. 2016 December ; 13(12): 980–992. doi:10.1080/15459624.2016.1200725.

Evaluation of standardized sample collection, packaging, and decontamination procedures to assess cross-contamination potential during *Bacillus anthracis* incident response operations

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Abstract

Sample collection procedures and primary receptacle (sample container and bag) decontamination methods should prevent contaminant transfer between contaminated and non-contaminated surfaces and areas during bio-incident operations. Cross-contamination of personnel, equipment, or sample containers may result in the exfiltration of biological agent from the exclusion (hot) zone and have unintended negative consequences on response resources, activities and outcomes. The current study was designed to: (1) evaluate currently recommended sample collection and packaging procedures to identify procedural steps that may increase the likelihood of spore exfiltration or contaminant transfer; (2) evaluate the efficacy of currently recommended primary receptacle decontamination procedures; and (3) evaluate the efficacy of outer packaging

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decontamination methods. Wet- and dry-deposited fluorescent tracer powder was used in contaminant transfer tests to qualitatively evaluate the currently-recommended sample collection procedures. *Bacillus atrophaeus* spores, a surrogate for *Bacillus anthracis*, were used to evaluate the efficacy of spray- and wipe-based decontamination procedures. Both decontamination procedures were quantitatively evaluated on three types of sample packaging materials (corrugated fiberboard, polystyrene foam, and polyethylene plastic), and two contamination mechanisms (wet or dry inoculums). Contaminant transfer results suggested that size-appropriate gloves should be worn by personnel, templates should not be taped to or removed from surfaces, and primary receptacles should be selected carefully. The decontamination tests indicated that wipe-based decontamination procedures may be more effective than spray-based procedures; efficacy was not influenced by material type but was affected by the inoculation method. Incomplete surface decontamination was observed in all tests with dry inoculums. This study provides a foundation for optimizing current *B. anthracis* response procedures to minimize contaminant exfiltration.

Keywords

Bacillus anthracis; cross-contamination; decontamination; sampling

Introduction

In late 2001, several letters containing spores of *Bacillus anthracis*, the etiological agent of anthrax, were distributed through the U.S. mail system. As a direct result, 22 people developed either cutaneous (N = 11) or inhalation (N = 11) anthrax; 5 of those with inhalation anthrax died. In addition, a number of buildings were contaminated and emergency response activities were conducted in numerous locations.[1–6] This process took several years to complete. Remediation costs following the “Amerithrax” incident were estimated at $320 million,[7] and the total cost of the incident, excluding economic impacts, was estimated at over $1 billion.[8,9]

Approximately 120,000 environmental samples were collected during the response to this incident.[7,10] Numerous samples collected from areas, or assets, outside the zone of primary contamination showed that the spores readily escaped the exclusion zone (i.e., hot zone or contaminated area). Exfiltration of spores potentially occurred via reaerosolization and airborne transport, transport on responder personnel, or insufficient decontamination procedures for items transported out of the exclusion zone such as personal protective equipment (PPE), equipment, or environmental sample containers.[11] The suspected case of cutaneous anthrax acquired by a laboratory worker who was processing Amerithrax samples also demonstrated the risks of handling samples associated with *B. anthracis*.[12]

In the years following the 2001 anthrax incident, numerous studies have been conducted to understand efficiencies of sampling procedures for spore collection from environmental matrices.[13–22] However, few studies have focused on the vulnerabilities of the field-collection procedures, particularly with regard to their potential for facilitating exfiltration of contaminants from the exclusion (hot) zone thereby contaminating assets not previously contaminated.[23] Two administrative controls used to prevent or reduce contaminant

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exfiltration from the exclusion zone are: (1) the use of vetted and standardized sample collection procedures which include the use of a two-person team: a support person that does not come into contact with potentially contaminated surfaces to be sampled and a sampler that collects the sample from a potentially contaminated surface; and (2) implementation of primary receptacle decontamination procedures prior to removal from the exclusion zone and outer packaging decontamination upon arrival at the receiving laboratory. Determining the vulnerabilities in the current procedures is imperative so that refinements, if necessary, can be made to the procedures to reduce the risk of cross-contamination and exfiltration.

The International Air Transport Association's (IATA) Dangerous Goods Regulations specify packaging for infectious substances affecting humans (UN 2814). Packaging must include an inner packaging comprised of a leak-proof primary receptacle (the sample container), a leak proof secondary packaging with absorbent material (for other than solid infectious substances), and a rigid outer packaging. Following the CDC protocol and historical practices, this translates into a primary receptacle consisting of a specimen cup and a resealable polyethylene plastic bag and an outer packaging of a polystyrene foam insulation inside a fiberboard box.

Decontamination of the sample container is recommended by the CDC in the contamination reduction (warm) zone when samples are removed from the exclusion zone. Secondary and outer packaging should not enter the exclusion or contamination reduction zones. The secondary and outer packaging should only be handled in a known clean environment and environmental samples should only be packaged after they have been properly decontaminated.

The objectives of the current study were to evaluate current biological sample collection and packaging procedures for potential sources of cross-contamination, and to determine the effectiveness of sample packaging (primary receptacle and outer packaging) decontamination procedures. Cross-contamination during sample collection was evaluated with dry- or liquid-deposited fluorescent tracer powder, using sample collection procedures recommended by CDC or the U.S. Environmental Protection Agency (EPA).[14,24–26] Primary receptacle packaging decontamination procedures recommended by CDC[26] and utilized by EPA[24] were evaluated on relevant packaging materials that were experimentally contaminated with *B. atrophaeus* spores by either liquid droplet or dry aerosol inoculation. Because of the concern that outer packaging may become contaminated when shipped during a response to a wide area release, this study also looked at the possibility outer packaging could be decontaminated by the receiving laboratory following the same methods. The findings of this study can be used to optimize field sample collection, packaging, and laboratory receiving procedures to reduce the risk of cross-contamination during future *B. anthracis* response activities.

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Materials and methods

Contaminant transfer tests

To qualitatively assess the potential for contaminant transfer from contaminated surfaces to sampling personnel and onto sample packaging materials, a long wave UV fluorescing melamine tracer powder (Risk Reactor, P/N PXT-07, Santa Ana, CA) in the size range of 5–15 μm was used as a *B. anthracis* spore simulant. Simulant was deposited onto slate laboratory bench material in a chemical fume hood by both dry and wet methods during separate tests. Dry deposition was accomplished using a bellows type pesticide powder duster (Southern Homewares, P/N 818947013256). To deposit the powder, the duster was inverted and the bulb squeezed, releasing about 0.020 g (determined gravimetrically) of powdered fluorescent tracer per actuation. For each test with dry tracer powder, the duster was actuated into an inverted plastic storage container ten times over an exposed surface area of approximately 2412 cm^2, resulting in an estimated surface concentration of about 0.082 mg/cm^2. For wet deposition, the tracer powder was suspended in 100 mL of 100% ethanol in a small plastic spray bottle that released about 0.019 g (determined gravimetrically) of tracer powder per sprayer actuation. For each test with wet tracer powder, the sprayer was actuated ten times into the same plastic storage container used for dry deposition over the same surface area, resulting in an estimated surface concentration of about 0.079 mg/cm^2. Wet or dry tracer powder was deposited and allowed to dry (approximately 10 min) or settle (approximately 10 min), respectively. For all wet and dry powder tests, a 645 cm^2 area in the center of the 2412 cm^2 area where powder was deposited was designated for performing the surface sample collection procedure (Figure 1). The area that received tracer powder was considerably (3.7 times) larger than the area designated for sampling in order to model sample collection in the hot zone, where contaminants are not visible to the sampler and thus may extend beyond the sample collection template. Surface samples were collected with 3M Sponge-Sticks using the procedures described previously[13,14,18] (Figure 2). Video and photography under visible or long-wave ultraviolet light were used to document procedural steps in which gloves came in contact with contaminated surfaces and to assess contaminant (tracer powder) transfer following execution of each sampling procedure.

The test matrix was designed to evaluate each procedural variation that differ between the CDC NIOSH website-published method,[26] the CDC NIOSH video published online,[25] and a recent inter-agency (EPA, Department of Homeland Security, Department of Defense) field test (Bio-Response Operational Testing and Evaluation; BOTE) sampling plan.[24] These differences include glove size (extra-large versus size appropriate), securing the sampling template with tape or holding it in place with one hand during sampling, and removing the template following the completion of sampling (EPA method), or leaving it in place (CDC method). These procedural differences are captured in the experimental plan (Table 1), and were varied in the current study as independent variables.

More specifically, over an existing pair of appropriately sized gloves, the sampler donned either extra-large nitrile gloves (EPA method) or large (size-appropriate for the sampler) nitrile gloves (CDC method), depending upon the test under study. A 10″ × 10″ paper

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sampling template was placed on the contaminated surface by the sampler and either taped down on two sides (CDC method), or left un-taped (EPA method for horizontal surfaces), as indicated in the test matrix. The area inside the template was then sampled with a 3M Sponge-Sticks according to the standardized CDC protocol. After sampling, the tip of the 3M Sponge-Stick was broken off by the sampler into a plastic specimen cup (Starplex 120 mL specimen cup, P/N 14-375-459, Fisher Scientific (straight walls); or VWR 133 mL specimen cup, P/N 25384-144 (tapered walls)) held by the support person. The sampler was right handed. When the template was taped down, the sampler did not touch the template with their left hand, however when no tape was used to secure the template, the sampler held the template in position with at least two fingers of their left hand during sampling (Figures 3E and 3F).

After each step in the sampling process, the sampler's gloved hands were placed in a light box and exposed to long wave UV light. Contamination on the sampler's gloves was documented with an HD video camera (Sam-sung HMX-F90) and a HD digital camera (Pentax K20). Pictures were also taken under normal light conditions to show where in the sampling process cross contamination may occur. The amount of cross contamination was not quantified, however, the magnitude of contamination was often apparent.

To assess the potential for contaminant transfer from contaminated support personnel to the primary receptacle, both extra-large and large gloves were intentionally contaminated (by touching glove fingertips to a surface laden with tracer powder) before executing the post-collection sample packaging procedures. While sample collection procedures, if followed explicitly, should preclude the possibility of the support personnel's gloves becoming contaminated, working in a contaminated zone often yields unforeseen circumstances. To this end, we sought to understand the potential consequences of the support person becoming contaminated and subsequently handling sample packaging materials. During these tests, specimen cups were capped, covered with Parafilm and packaged in plastic bags (the primary receptacle) as prescribed in the CDC protocol.[25]

Bacterial spore preparations and inoculation methods

Both liquid and dry (aerosolized) preparations of *B. atrophaeus* (American Type Culture Collection (ATCC) 9372; formerly *Bacillus subtilis* var *niger* and *Bacillus globigii*) were used as a surrogate for *B. anthracis*. Wet and dry inoculation methods represent two contamination scenarios that may occur during a biological incident. Liquid spore preparations were obtained from Yakibou, Inc. (formerly Apex Laboratories) in deionized water at a concentration of about 6×10^8 colony forming units (CFU) per mL and were diluted with 10% ethanol (v/v) to a concentration of about 4×10^5 CFU per mL for inoculation onto test materials. Dry spores were obtained from the U.S. Army's Dugway Proving Grounds and were prepared[21,27] before being loaded into pressurized metered dose inhalers (MDIs) by Cirrus Pharmaceuticals (Durham, NC) as reported previously.[28] The MDIs delivered a concentration of approximately 2×10^7 spores per 50 μL actuation.

For dry deposition tests, the center-most 929 cm^2 portion of clean, dry, sterile materials were inoculated with aerosolized *B. atrophaeus* spores using procedures described previously.[28] Briefly, the MDI was loaded into an aluminum actuator positioned above the test material

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coupon at the top of a sealed pyramid-shaped chamber and actuated once to release the aerosolized spores. Following release, test material coupons remained sealed and undisturbed for 18–21 hr to allow gravitational settling of the spore inoculum. For wet spore inoculations, 1 mL of the liquid inoculum was deposited onto material coupons as a series of ten 100 μL droplets using a micropipette. The inoculum was allowed to dry 18–21 hr prior to test treatment initiation. This inoculation method is similar to those described previously.[14,18] The targeted recovery from positive controls was 2×10^5 and 1×10^7 CFU for liquid and dry inoculums, respectively.

Sample package material decontamination tests

Sample package material decontamination tests were conducted to evaluate the efficacy of two currently-recommended decontamination methods, sporicidal wipe-based and sporicidal spray-based approaches.[24,26] Test materials consisted of 1264.5 cm^2 pieces of corrugated fiberboard (outer packaging from Thermosafe EPS, P/N 352; Arlington Heights, IL), polystyrene foam (outer packaging insulating container from Thermosafe EPS, P/N 352; Arlington Heights, IL), and polyethylene plastic (primary receptacle [Ziplock 3 gallon bag] from S.C. Johnson & Son, P/N 255927, Racine, WI) (Figures 4A–C). Stainless steel (16-gauge, 316 stainless; Dillon Supply, Raleigh, NC) coupons (Figure 4D) of the same size were used as positive control reference samples to verify inoculation procedures. Prior to inoculation, the stainless steel reference coupons were steam sterilized via autoclave at 121°C while the packaging materials were sterilized with ethylene oxide, to maintain material integrity, according to manufacturer's instructions (Anderson EO Gas AN333 system, Haw River, NC).

The decontamination efficacy of sporicidal bleach wipes (Clorox Healthcare Bleach Germicidal Wipes) and pH adjusted bleach (PaB) spray-based decontamination procedures was investigated for both contamination methods (wet or dry inoculum), and all three test materials. Tests were conducted under ambient laboratory conditions, with the temperature and relative humidity monitored but not controlled (21–24°C; 25–55% RH). Five test replicates, three positive control replicates, and one blank were utilized for each combination of material type, decontaminant, and inoculation method.

For the sporicidal wipe decontamination tests, procedures were adapted from ASTM E2896-12,[29] as presented previously.[30] For each test replicate, bleach wipes were folded in half, then half again, and the coupon was first wiped in the horizontal direction, back and forth, until the entire surface had been wetted. Next, the wipe was folded in half again and the surface wiped in the vertical direction until the entire surface had been covered. Finally, the bleach wipe was folded a third time and the surface wiped diagonally beginning at the upper left corner.

For the spray-based decontamination procedure, PaB solutions were prepared daily by first diluting the bleach (Clorox Healthcare Concentrated Bleach) containing 8.25% sodium hypochlorite 2:1 with deionized (DI) water. The diluted bleach was then mixed with DI water and 5% (v/v) acetic acid (Fisher Scientific, P/N S25623A) to result in a ratio of 1:8:1 (bleach:water:acetic acid), respectively, having a resulting pH of about 6.8 (confirmed with a pH meter) and a free available chlorine concentration of about 6530 mg/mL as measured

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using a HACH digital titrator (Hach Company, P/N 26869-01, Ames, IA) loaded with 2.26 N stabilized sodium thiosulfate. The solution was adjusted as necessary to obtain the target pH of 6.8. Following preparation, the PaB was transferred into a high density polyethylene hand sprayer (Flo-Master, Model 1985VI, Lowell, MI), pressurized to 5 psi by hand-pumping, and sprayed onto horizontally oriented coupons from a distance of approximately 30 cm for 5 s in a zig-zag pattern to fully wet the surface. Only one application of PaB was administered. Surfaces remained visibly wetted for at least 10 min, in accordance with CDC recommendations.[26] Surfaces were not mechanically dried with a towel as the CDC guidance is not prescriptive in the drying method.

Following both decontamination methods, coupons remained undisturbed for an average of 21 hr to allow drying before sampling with pre-moistened 3M Sponge-Sticks (3M, St. Paul, MN, P/N SSL10NB) using the standardized CDC protocol for sampling nonporous surfaces.[14,18,26] Stainless steel reference coupons, positive control (non-decontaminated material coupons), and test coupons were all sampled using the CDC protocol, on the same day. *B. atrophaeus* spores were recovered from the 3M Sponge-Sticks using the procedures described in the CDC's national validation study.[14] inoculated in triplicate onto trypticase soy agar with an Autoplate spiral plating system (Advanced Instruments, Inc.; Grove, IL) and incubated overnight at 35 ± 2°C. CFUs were then enumerated with a Q-Count automated colony counter (Advanced Instruments, Inc.). Negative controls and samples with fewer than 30 CFU per plate were filter-plated by collecting 1 mL and 9 mL of the sample extract onto Pall 0.45 μm pore-size microfunnel filters (P/N 4804). The filters were placed collection side up onto TSA plates, incubated overnight at 35 ± 2°C and then manually enumerated. Recovery data are reported as Log_{10} CFU, decontamination efficacy data are reported as Log Reduction in total recovery (Log_{10} positive controls – Log_{10} experimental), and were reduced as described previously.[31] Statistical significance was assessed using a p-value threshold of 0.05.

Results

Contaminant transfer

The tracer powder was readily visible under long-wave ultraviolet light (Figure 1). Observation of hand positions relative to experimentally contaminated surfaces indicated that gloved hands have a high potential to contact surfaces during sampling procedures (Figure 3). The oversized gloves had a greater potential to contact the surface during sampling. Securing the sampling template to the surface can result in glove contamination, both from the act of taping and from holding the template in place during taping (Figures 3C through 3F). Not using tape to secure the template to the surface can result in contamination transfer to gloves during sampling. This was due to the need for one hand to hold the template in place during sample collection and therefore at higher risk of contacting the contaminated surface (Figures 3E and 3F). Irrespective of glove size, there is potential to cross contaminate sampling personnel during sponge head snap-off into the specimen cup (Figures 3I and 3J). Aerosolization of the wetting agent was observed in the current study during snap-off of the sponge head. This could result in external contamination of the specimen cup and other surfaces. This occurred most frequently when the sampler struggled

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to break the sponge head from the stick. Trials with two different specimen cups (a straight side-wall cup and a tapered-wall cup) were conducted to determine if cup type affected this occurrence. From our qualitative observations, we concluded that tapered-wall specimen cups eased sponge head snap-off and thereby reduced the risk of wetting agent aerosolization during snap-off.

Observation of gloves following each procedure showed that template taping and template removal resulted in contaminant transfer from the surface to gloved fingers, for both dry and wet deposition methods (Figure 5). Tests in which the post-collection sample packaging procedures were conducted following purposeful contamination of the support person indicated that contaminants can be transferred to sample containers (Figure 6). There were no observed differences between wet and dry tracer deposition methods with respect to cross-contamination frequency or magnitude.

Sample package material decontamination tests

The results of the sample package material decontamination tests are shown in Table 2. Recoveries (mean ± std. dev) from positive control reference coupons were 5.5 ± 5.3 Log_{10} CFU (3.5 ± 1.9 × 10^5 CFU) for tests with liquid inocula and 7.3 ± 7.0 Log_{10} CFU (2.2 ± 1.1 × 10^7 CFU) for tests with aerosol inocula (Table 2). For the liquid inoculated test coupons, both decontamination methods were equally effective at reducing the spore loads. There was no statistically significant difference between decontamination methods ($p = 0.383$) or materials ($p = 0.327$) (t-test). Only one post-decontamination sample yielded viable spores during tests with liquid inoculums. Viable spores (2 CFU) were recovered from one of five replicates for the sporicidal wipe-decontaminated polyethylene plastic sample. For the aerosol deposition samples, none of the conditions tested yielded complete kill (i.e., viable spores were recovered following decontamination during all tests). For these tests there was a statistically significant difference ($p < 0.001$) between the sporicidal wipe and PaB spray decontamination methods, with the wipe achieving higher decontamination efficacies. For the tests utilizing the sporicidal bleach wipe decontamination method, the average post-decontamination recoveries (log_{10} CFU) were, 1.6 ± 1.6, 1.7 ± 1.0, and 0.6 ± 1.2 for corrugated fiberboard, polystyrene foam, and polyethylene plastic, respectively. For the PaB spray-based decontamination method, the average post-decontamination recoveries (log_{10} CFU) were, 4.4 ± 0.5, 4.8 ± 0.6, and 5.4 ± 0.2 for corrugated fiberboard, polystyrene foam, and polyethylene plastic, respectively.

Discussion

During past biological emergency response investigations, exfiltration of contaminants from the exclusion zone has been documented.[23,32] This can have serious consequences, including increasing sampling and decontamination requirements, increasing overall operation costs, and increasing the risk of exposure of unprotected workers or civilians. Current sample collection and primary receptacle decontamination procedures have been designed to optimize sample integrity while minimizing the risk of contaminant transfer from the exclusion zone to areas previously not contaminated. Proper laboratory practices and the use of biological safety cabinets during sample processing and analytical methods

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also reduce the risk of laboratory contamination and worker exposure.[33] Nevertheless, few studies have systematically evaluated the two main administrative controls used to minimize cross-contamination: sample collection procedures and primary receptacle decontamination prior to removal from the exclusion zone. The current study utilized a systematic approach to evaluate the currently-recommended sample collection and primary receptacle decontamination procedures for cross-contamination potential, in order to improve these procedures. In addition, outer packaging materials were also tested following current decontamination recommendations to see if receiving laboratories can use the procedures in the event of suspected cross-contamination during shipment. Tests were also conducted to determine cross-contamination potential in the unlikely scenario in which the support person's gloves become contaminated. For these tests, the support person's gloves were intentionally contaminated with fluorescent tracers prior to executing sample collection and packaging procedures.

It has long been recognized that fomites (inanimate objects) can play a significant role in contaminant transfer and thus pathogen transmission.[34,35] Contaminants acquired by touching contaminated surfaces can be subsequently transferred to other surfaces or individuals.[34] Further, contaminants can be distributed and redistributed numerous times, as evidenced by a study on multi-generational contamination of letters containing *Bacillus* spores.[36] The magnitude of contaminant transfer is determined by many factors, including contaminant characteristics, surface characteristics, particle-surface interactions, contaminant load, barrier or PPE type, adherence to aseptic techniques, contact force or mechanism, and environmental conditions.[37–39] Although not quantitative in nature, the current study sought to identify steps within standardized procedures that may lead to downstream contamination of laboratories, assets, or previously uncontaminated areas. Identification of such vulnerabilities, with subsequent refinement in procedures, are essential for emergency response activities involving high-consequence pathogens such as *B. anthracis* spores. In the current study, fluorescent tracer powder was used during sample collection procedures to identify steps that may facilitate cross-contamination. Such tracers have been used in previous studies for qualitative identification of mechanisms and rates of cross-contamination.[40] The results of the current study offer insight into the procedural steps that pose the greatest potential for cross-contamination. Refinement of the most vulnerable steps may mitigate the risk of cross-contamination. However, it is important to note that only one type of fluorescent tracer powder was utilized in the current study, and that the physical characteristics of this tracer may be different than those of *Bacillus* spores. Others have shown that tracer selection can influence results and conclusions.[37,41] Nonetheless, tracers provide a simple means to rapidly identify procedural steps in which contaminants may be transferred. Since transfer of contaminants was by direct contact with contaminated surface, it is unlikely that another tracer powder would yield differing results.

In reviewing the photographic data from the current study, several procedural steps or practices were identified as potentially increasing the risk of cross-contamination. The prescribed use of extra-large gloves during sampling, without regard to sampling personnel's hand size, may increase the potential for contamination of gloves and subsequently any item touched thereafter. Keen hand position awareness and secure fitting gloves (size appropriate for specific sampling personnel), increase dexterity and decrease the amount of glove

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surface area available to contact contaminated surfaces. Pre-assembly of sampling kits prior to exclusion zone entry could be problematic as the hand size of sampling personnel is likely unknown at the time of kit assembly. Gloves could be packaged separately from supplies for sampling operations, and each individual could deploy with a size-appropriate supply of gloves.

Both securing the template with tape and not securing the template (requiring one hand to secure it while sampling) increased the risk of cross-contamination. Templates should be taped only when necessary, such as when sampling vertical surfaces. Adhering double-sided adhesive strips on the back of templates, prior to exclusion zone entry, may provide an easy means for securing templates without the use of tape, and relieve the sampler from needing to hold the template in place with a gloved hand. However, removal of the paper backing from adhesive strips while wearing gloves may prove difficult. Similarly, the CDC-prescribed taping of the template (Figure 3) was challenging to execute while wearing gloves. Removal of templates after sampling procedures also poses unnecessary risks of cross-contamination. Templates should be left in-place until the area is determined safe for re-entry.

The type of specimen cup used may contribute to contamination transfer. During the current study, it was observed to be much easier and required less force to break off the 3M Sponge-Stick tip if the walls of the specimen cup were sloped rather than perpendicular to the bottom of the cup. Struggling with tip removal post-collection could potentially generate aerosol droplets from the 3M Sponge-Sticks, or even result in dropped samples. Sample bags, such as whirl-pak or twirl-em, are commonly used as spill-proof containers for liquid samples and may allow ease of sponge head detachment compared to either of the specimen cup options. Shipping regulations for hazardous or infectious materials posted by the U.S. Department of Transportation and the International Air Transport Association should be reviewed prior to final selection of sample package and containment vessels.

Both wet and dry contamination mechanisms are possible during a *B. anthracis* incident, whether by the initial contaminant dispersion mechanism or by contaminant redistribution during sampling and decontamination activities. Accordingly, the current study evaluated the efficacy of decontamination methods against spores deposited by both wet (droplet) and dry (aerosol) mechanisms on three common sample packaging materials. The decontamination test results suggest that material type does not significantly affect efficacy, for the materials tested. Inoculation method and decontamination method (for the dry inoculum only) had more impact on efficacy than did material type for the materials included in this study. Previously, sporicidal wipes were shown to be effective on numerous material types, such as stainless steel, glass, composite epoxy, painted drywall, and low-density polyethylene plastic.[30]

Due to the significant difference between the liquid (10^5) and dry (10^7) inoculum titers, the authors urge caution when comparing decontamination efficacy results across inoculum types. In general, tests utilizing liquid inoculums resulted in higher decontamination efficacies than tests where the same methods were performed on surfaces receiving the dry inoculum. Indeed, other studies have noted that differences in decontamination efficacy can

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been attributed to inoculation method.[42] Because liquid spores were deposited in ten discrete spots of 100 μL across a coupon in a predictable pattern rather than evenly distributed, it is possible that: (1) the location of contamination was known and therefore unintentionally targeted during the decontamination procedure; (2) physical removal of contaminants is more efficient when the liquid inoculum is dried into small, discrete locations rather than deposited as dry particles over the entire coupon surface; and (3) the actual amount of inoculated surface area was significantly smaller for liquid tests (the area under ten small droplets), and thus decontamination procedures had a much higher probability of treating 100% of the contaminated area. For example, if a small amount of coupon surface area was inadvertently neglected during the decontamination treatment, the impact on viable spore recovery could be much greater for the tests with dry inoculums. Liquid droplet contamination of sample packages is a realistic scenario in a field situation, possibly occurring following liquid spray decontamination procedures. In addition, contamination of sample packaging by liquid droplets could potentially occur during personnel and equipment decontamination line procedures, at the boundary between the exclusion and support zones. Liquid wash-down of equipment and personnel is a common decontamination line procedure during *B. anthracis* response operations. During such activities, contaminants may be redistributed by sprays, scrub-brushes, or runoff water.

Bleach wipes demonstrated higher decontamination efficacies than PaB spraying for aerosol-inoculated test coupons. This could be due to both the effectiveness of the decontaminant and the dual action of chemical inactivation and physical removal of spores during the wipe-based method. The contribution of physical removal, without spore inactivation, was not determined for the sporicidal wipe method during this study nor was the interaction between chemical and physical forces. This study did not look at the efficacy of the spray-based method used in conjunction with mechanical drying of the surface compared to air drying. It is unclear what contributions the physical removal provided through mechanical drying would have on the PaB spray-based method's efficacy. Alternately, incomplete coverage of contaminated surfaces using the PaB spray method, could permit viable spores to survive decontamination treatment. Spore hydrophobicity may also affect spore movements with water and decontaminant (cluster during spray-based application), and these effects may differ between liquid and dry inoculums thereby contributing to disparities in decontamination efficacies. While further tests should be conducted to determine the effects of the contamination mechanism on decontamination efficacy, the current data suggest that wipe-based procedures for sample containers are superior to spray-based methods. These results corroborate previous studies that demonstrated the effectiveness of wipe-based surface decontamination approaches on steel, glass, composite epoxy, dry wall, and low-density polyethylene.[30,43] Wipe-based sample container decontamination procedures are also more feasible to conduct in restrictive protective gear, and take less time to execute. These factors (time and ease of use) weigh heavily on method selection for real-world response operations.

Conclusions

The current study sought to gain an understanding of cross-contamination potential when using the currently-recommended sample collection procedures, primary receptacle

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decontamination procedures, and the potential for outer packaging decontamination at the receiving laboratory. In addition, this study sought to understand cross-contamination from the unlikely event that the support person becomes contaminated during the sampling procedure. Evaluation and revision of these procedures is critical for ensuring sample integrity and preventing cross-contamination during sampling operations following a biological incident. The following conclusions can be drawn from the test results.

- Sporicidal wipe-based package decontamination procedures achieved higher decontamination efficacies than spray-based procedures (aerosol inoculum tests).
- Decontamination of dry-deposited spore inoculums was more difficult than wet-deposited spores (complete kill was not achieved during tests with dry-deposited spores).
- To ease sponge head snap-off, tapered-side specimen cups are preferred over straight-side specimen cups for 3M Sponge-Stick primary containment.
- Size-appropriate gloves resulted in less contaminant transfer by sampling and support personnel than extra-large gloves.
- It is suggested that templates be taped to the surface only if necessary, and remain in place after sampling.

Acknowledgments

Funding

This research was supported in part by an appointment to the Research Participation Program for the U.S. Environmental Protection Agency, Office of Research and Development, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and EPA.

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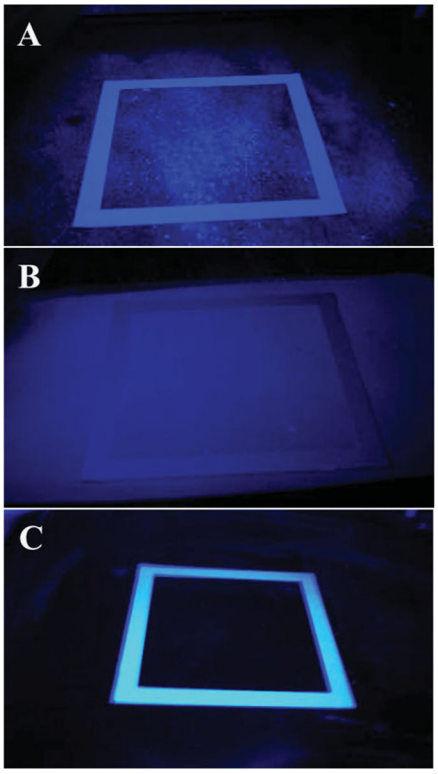

Figure 1.

Representative photographs of surfaces where sampling procedures were rendered following wet deposition (A) or dry deposition (B) of fluorescent tracer powder. Fluorescent tracer powder was deposited onto 2412 cm^2 section of a laboratory bench surface, a paper template demarcating a 645 cm^2 area for sampling was subsequently placed in the center of that area. Panel C is a representative photograph of the area prior to tracer deposition. Photographs were taken under long-wave UV light.

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1		Place template on surface to be sampled
2		Tape to secure to surface (if the template is not taped to the surface, it will be necessary to place two fingers from one hand on template to hold in place during sampling)
3		Sample horizontally using overlapping horizontal s-strokes to wipe entire surface inside template with one flat side of the 3M Sponge-Stick
4		Turn 3M Sponge-Stick to the opposite flat side and sample vertically using overlapping horizontal s-strokes to wipe entire surface inside template
5		Turn 3M Sponge-Stick to an edge and sample diagonally using overlapping horizontal s-strokes to wipe the entire surface inside template
6		Finally, use the tip of the 3M Sponge-Stick to sample the entire perimeter inside the template
7		Break tip of 3M Sponge-Stick into a sterile specimen cup

Figure 2.
CDC standardized 3M Sponge-Sticks sampling procedure.

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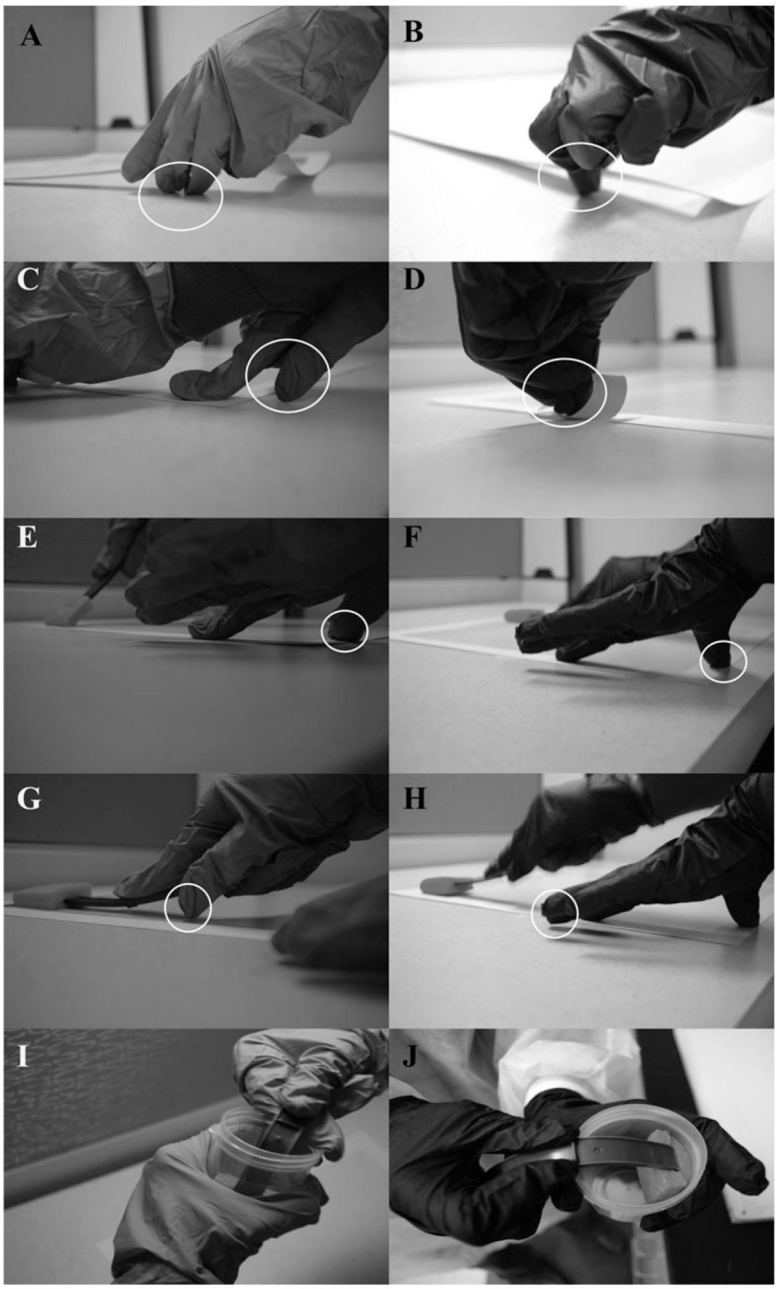

Figure 3.

Representative photographs of hand positions and potential cross-contamination points during sample collection with the 3M Sponge-Stick samplers. Photographs depict placement of the template where the sampler had donned extra-large (A) or size-appropriate gloves (B), taping the template to the surface using extra-large (C) or size-appropriate gloves (D), holding the template during sampling using extra-large (E) or size-appropriate gloves (F), conducting the sample collection procedure using extra-large (G) or size-appropriate gloves (H), and breaking the sponge collection head into the specimen cup using extra-large (I), or size-appropriate gloves (J).

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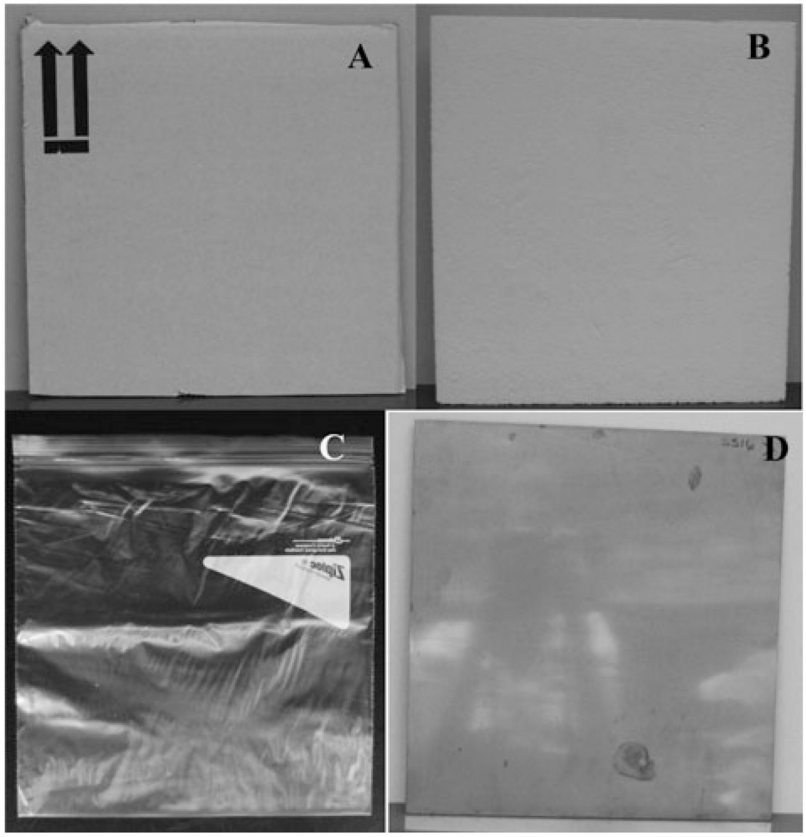

Figure 4.
Photograph of representative coupons utilized during sample package material decontamination tests. Coupons consisted of corrugated fiberboard (A), polystyrene foam (B), both 35.6 cm × 35.6 cm, or polyethylene plastic bags (C). Stainless steel coupons (D) were utilized as inoculation controls.

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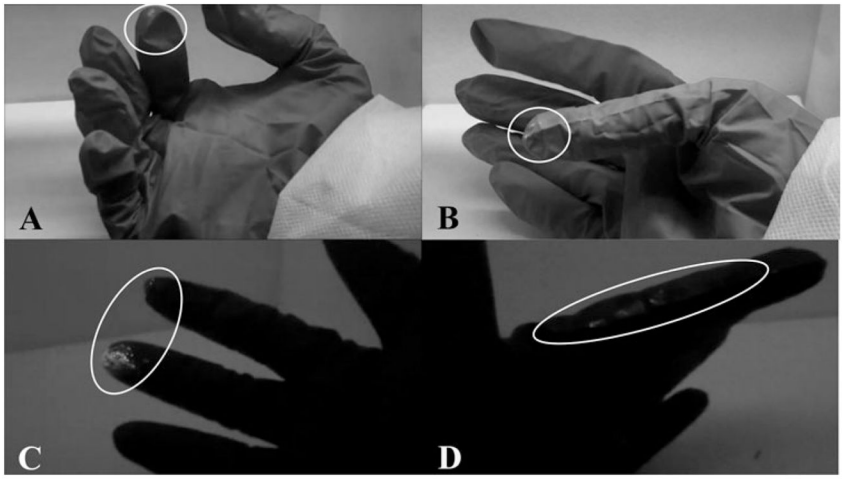

Figure 5.

Representative photographs of glove contamination resulting during 3M Sponge-Stick sampling procedures. Photos depict contamination on glove fingertips following dry-deposition sampling with extra-large gloves (A), contamination on extra-large glove fingertips following taping of the template (dry deposition) (B), contamination on size-appropriate glove fingertips following template removal (dry deposition) (C), and contamination on size-appropriate glove fingers following template removal (wet deposition) (D).

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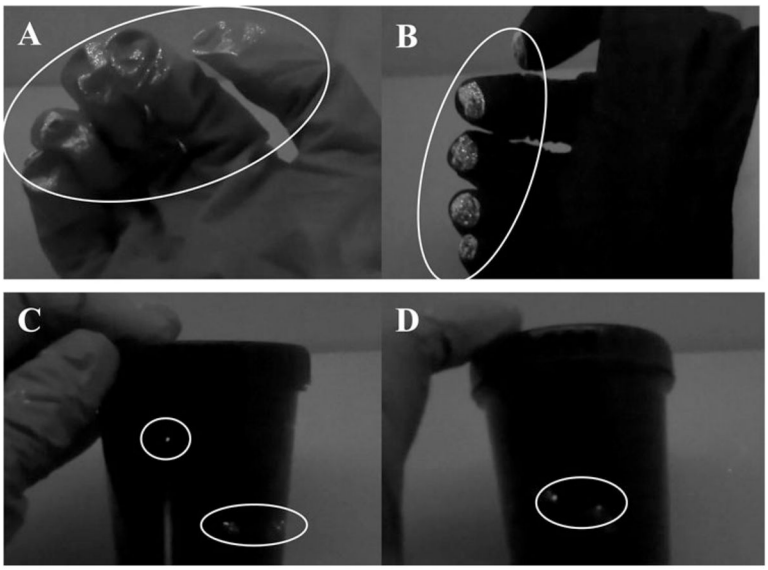

Figure 6.

Representative photographs of cross-contamination resulting from the contamination of the support person during packaging of 3M Sponge-Sticks. Gloves were purposefully contaminated prior to beginning the packaging procedure to determine the potential for contamination of sample containers. Photos depict contamination on glove fingertips following purposeful contamination while wearing extra-large gloves (A), contamination on size-appropriate glove fingertips (B), contamination specimen cups following closure with extra-large gloves (C), and size-appropriate gloves (D).

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Table 1

Summary of contaminant transfer test variables. Tracer powder was deposited onto a larger area (2412 cm^2) from which surface samples (645 cm^2) were collected. Tests were conducted with either wet- or dry-deposited powder in order to simulate two differing contamination scenarios. Steps that differed between the EPA and CDC procedures were varied in the eight tests conducted to determine the effect on cross-contamination of gloves and sample containers.

Test	Deposition	Gloves	Template	Template Removal
1	Dry	Extra Large, Powder Free, Nitrile	Taped	Not removed
				Removed & disposed
2			Not Taped	Not removed
				Removed & disposed[a]
3		Large, Powder Free, Nitrile	Taped	Not removed[b]
				Removed & disposed
4			Not Taped	Not removed
				Removed & disposed
5	Wet	Extra Large, Powder Free, Nitrile	Taped	Not removed
				Removed & disposed
6			Not Taped	Not removed
				Removed & disposed[a]
7		Large, Powder Free, Nitrile	Taped	Not removed[b]
				Removed & disposed
8			Not Taped	Not removed
				Removed & disposed

[a] EPA method.

[b] CDC method.

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Table 2

Summary of results from the sample package material decontamination tests. Mean Log_{10} recoveries of decontaminated and positive control test materials, and mean decontamination efficacies (Log_{10} reduction) are presented for each test. Five replicate test samples, three replicate positive control samples, and one blank sample were utilized for each condition.

			Recovery (Log_{10} CFU)[a]		
Inoculum	**Decontaminant**	**Test Material**	**Positive Control[b]**	**Test Coupon[c]**	**Decontamination Efficacy (Log_{10} Reduction)**
Liquid	pH-adjusted bleach spray	Corrugated Fiberboard	5.2 ± 0.0	0.0 ± 0.0	5.2 ± 0.0
		Polystyrene Foam	5.0 ± 0.1	0.0 ± 0.0	5.0 ± 0.1
		Polyethylene Plastic	5.2 ± 0.0	0.0 ± 0.0	5.2 ± 0.0
	Sporicidal bleach wipe	Corrugated Fiberboard	4.6 ± 0.1	0.0 ± 0.0	4.6 ± 0.1
		Polystyrene Foam	4.7 ± 0.4	0.0 ± 0.0	4.7 ± 0.2
		Polyethylene Plastic	5.6 ± 0.1	0.1 ± 0.1	5.5 ± 0.1
Aerosol	pH-adjusted bleach spray	Corrugated Fiberboard	7.1 ± 0.1	4.4 ± 0.5	2.7 ± 0.2
		Polystyrene Foam	6.9 ± 0.5	4.8 ± 0.6	2.0 ± 0.4
		Polyethylene Plastic	7.3 ± 0.1	5.4 ± 0.2	2.0 ± 0.1
	Sporicidal bleach wipe	Corrugated Fiberboard	7.2 ± 0.2	1.6 ± 1.6	5.6 ± 0.7
		Polystyrene Foam	6.6 ± 0.4	1.7 ± 1.0	4.9 ± 0.5
		Polyethylene Plastic	7.6 ± 0.0	0.6 ± 1.2	7.0 ± 0.6

[a] Data are expressed as the log of the mean ± one standard deviation of the number of viable spores (CFU) recovered.

[b] Positive controls were inoculated, not decontaminated coupons of actual test materials.

[c] Test coupons were inoculated, decontaminated coupons.